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(54) Title: NEW BIOPOLYMERS OBTAINED BY SOLID STATE IRRADIATION IN AN UNSATURATED GASEOUS ATMOSPHERE
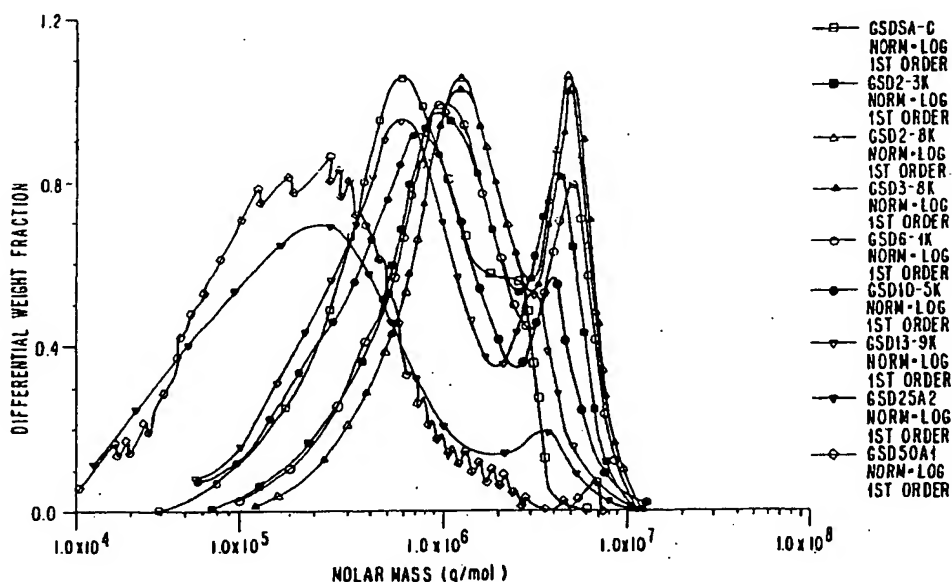
(57) Abstract: Disclosed is a method for modifying naturally occuring biocompatible biopolymers of plant and animal origin by subjecting same to ionizing radiation in the presence of a mediating gas, typically acetylene to enable one to selectively enhance and modify one or more of the physiochemical properties of the starting materials which have a wide range of uses in medicine, food technology and other industrial applications. Notwithstanding the modifications, the biocompatibility of the biopolymer remains unchanged and no new or additional functional groups are introduced into the starting biopolymer.

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NEW BIOPOLYMERS OBTAINED BY SOLID STATE IRRADIATION IN AN UNSATURATED GASEOUS ATMOSPHERE

5   Technical Field

The invention relates to a range of new products; processes for preparing them from dry biological polymers (biopolymers) using ionizing radiation in the solid - non fluid state in the presence of an unsaturated gas under specified reaction conditions and the uses thereof.

10   Background Art

It is known in the art to subject certain types of polymeric materials to irradiation in order to achieve a number of different goals, although to our knowledge it is not known in the art to subject such biopolymers to high energy irradiation in the presence of a mediating gas, e.g., acetylene, in order to modify the biopolymer so as to enhance its

15   properties in one or more respects. The following U.S. patents: 3,215,634 (Walker); 4,024,073 (Shimizu); 4,716,224 (Sakurai); 4,746,514 (Warne); 4,987,222; (De Ambrosi); and 5,376,692 (Park); and published foreign application WO 96/03147; (Fidia, S.p.A) are of interest, but are not significantly relevant. For example, none of the art teaches irradiation of polymeric materials in the solid state, including Warne ('514) who does use an ethylenically

20   unsaturated compound, which is not a gas.

Sakurai ('224) teaches the cross-linking of hyaluronic acid with polyfunctional epoxy compounds under certain conditions, none of which teach the use of ionizing radiation/unsaturated [alkenic or alkynic] gases.

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Walker ('634) and Shimizu ('073) also disclose the use of various chemical cross-linking agents for preparing cross-linked polysaccharide products.

De Ambrosi ('222) discloses the controlled preparation of low molecular

30   weight glucosaminoglycans by depolymerizing high molecular weight glucosaminoglycans using γ radiation.

Warne ('514) teaches away from the invention. Warne discloses the preparation of cross-linked hydrogels by subjecting a polysaccharide, specifically, nothing of

35   higher molecular weight than a pentasaccharide, to ionizing radiation in the presence of an ethylenically unsaturated compound (but not a gas) having at least one hydrophilic group.

Park ('692) discloses proteins, e.g., albumin, that are functionalized so that when the albumin is bonded to a blood compatible substrate, and after treatment with radiation, free radicals formed on both the protein and the substrate chemically bind to one another. This reference does not teach or suggest cross-linking the polymers by using ionizing radiation in the presence of an unsaturated gas which forms part of the cross-link.

Fidia (PCT application no. WO 96/03147) teaches the synthesis of chemical gels from polyelectrolyte polysaccharides, including HA and HA-benzyl ester by γ-irradiation, preceded by functionalizing to introduce olefinic bonds, into the structure thereof. The only functionalizing agent disclosed is glycidyl acrylate. Other, less relevant foreign patents such as EP 000 038 426; JP 360,143,991; JP 363,301,234; JP 401,118,529; DE 004,123,889; DE 004,124,338; and JP 406,073,102 are noted.

Non-patent literature relating to the subject matter of this invention, particularly, certain studies conducted on some of the starting materials used herein and the effect of ionizing radiations on uncharged polysaccharides (such as starch and cellulose) and on polyelectrolyte polysaccharides (such as hyaluronic acid and its cross-linked derivative hylan, alginates, heparin etc) is to induce degradation, with main chain scission leading to a decrease in molecular weight and viscosity are discussed in:

The effect of sterilizing doses of γ-irradiation on the molecular weight and emulsification properties of gum arabic; Blake, et al, Food Hydrocolloids 1988, Vol.2 No.5, p.407-415; The effects of radiation on carbohydrates (Phillips, G., Chapter 26 pages 1217-1297 in "The Carbohydrates", second edition. (Eds. Ward Pigman/Derek Horton), Academic Press Inc. New York, 1980); Free radical formation and degradation of cellulose by ionizing radiations. (Nakamura et al. Polymer Photochemistry, 1985, 6, 135-159); Photochemistry and radiation chemistry of cellulose
(Phillips et al. Cellulose Chemistry and Its Applications 1985, 290-311); Radiation effects on the biological activity and molecular weight parameters of heparin. (Edwards et al. Carbohydrate Polymers, 1985, 5, 473-478); The radiation-induced degradation of hyaluronic acid. (Deeble et al. Radiat.Phys.Chem. 1991, Vol.37, No.1, 115-118); Susceptibility of Connective Tissue: Biomaterials to Radiation. (Phillips et al. Journal of Korea Biomaterial Research Institute, Vol.1, No.1, August 1991, p.92); The enhanced stability of the cross-linked hylan structure to hydroxyl radicals compared with the uncross-linked hyaluronan. (Al-Assaf et al. Radiat. Phys.Chem. 1995, Vol 46, 207-217); Identification of radicals from hyaluronan (hyaluronic acid) and cross-linked derivatives using electron paramagnetic resonance spectroscopy. (Al-Assaf et al. Carbohydrate Polymers, 1999, Vol.38, 17-22); The

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role of the proteinaceous component on the emulsifying properties of gum arabic. (Randall et al. Food Hydrocolloids, 1988, 2, No.2, 131-140); Structural and chemical properties of gum arabic. Their practical impact. (Phillips et al. Proceedings Gum Arabic Symposium, ZDS, Solingen, Germany, June 6-8, 1988); The influence of structure and technology on gum

5   arabic functionality. (Phillips, G., Supplement to Food Review February/March, 1988, pp.64-68); Fractionation and characterization of gum from *Acacia senegal.* (Randall et al. Food Hydrocolloids, 1989, Vol.3, No.1, p.65-75); The molecular characterization of the polysaccharide gum from *Acacia senegal.* (Osman et al. Carbohydrate Research, 1993, 246, pp. 303); The Classification of Natural Gums. Part III. *Acacia senegal* and Related Species

10   (Gum Arabic); (Jurasek et al. Food Hydrocolloids, 1993, Vol.7, No.3, pp. 255-280); Acacia gum (Gum Arabic): a nutritional fibre; metabolism and calorific value. (Phillips, G., Food Additives and Contaminants, 1998, Vol. 15 No.3, 251- 264); and. A review of recent developments on the regulatory, structural and functional aspects of gum arabic. (Islam et al. Food Hydrocolloids, 1997, Vol 11 (4), pp 357-365). Fractionation and characterization of

15   gum from *Acacia Senegal.* (Randall et al. Food Hydrocolloids, 1989, Vol.3, pp.65-75) The molecular characterization of the polysaccharide gum from *Acacia senegal.* (Osman et al. Carbohydrate Research, 1993, 246, pp. 303); The Classification of Natural Gums. Part III. *Acacia Senegal* and Related Species (Gum Arabic) (Jurasek et al. Food Hydrocolloids, 1993, Vol.7, No.3, pp. 255-280).

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Disclosure of the Invention

The invention provides an extremely broad category of new biopolymers having dramatically improved properties in comparison with the starting biopolymers. The molecular weights of these materials can be increased in a controlled manner to provide new

25   physical and chemical functionalities (for example emulsification and water binding). Aqueous solutions of the new products can be produced with literally any desired viscosity and/or viscoelasticity. The biopolymers can be converted into new hydrophilic gels (hydrogels) of defined particular size and having specified micromechanical properties. The changes can be accomplished without the introduction of new chemical substituents, and

30   hence, the new materials retain the inherent biocompatibility of the starting, or parent biopolymer. One or more different biopolymers can be used together in the process to yield new bio-copolymers.

As used herein the term biopolymer and biological polymer is understood to

35   mean a polymer derived from a biological source, whether plant, including microorganisms or animal.

The biopolymers contemplated by the invention comprise unsubstituted biopolymers extending over the entire field of plant and animal derived polysaccharides, whether charged or uncharged, as well as proteins directly derived from animal connective tissue sources such as collagen, gelatin, and from human and animal products, such as casein, combinations of one or more such polysaccharides with one or more proteins of plant origin - - such as arabinogalactan proteins, biological tissues and materials derived therefrom used for tissue replacement and transplantation, either finished or partially finished and which are made or formed from one or more of such biopolymers or combinations thereof with the other aforesaid materials. The biopolymers to be treated to form the new materials according to the process of the invention do not need to be modified in any way prior to treatment, for example, by introducing any functionalizing groups which might, in other processes be necessary to activate the biopolymer or make it more reactive.

Illustrative examples of the biopolymers contemplated by this invention include:

acacia plant exudates, such as *acacia senegal* and *acacia seyal*, representing the arabinogalactan proteins present in all plants; dextran and related bacterial polysaccharides; chemically modified polysaccharides such as carboxymethyl cellulose; gelling polysaccharides from either bacterial origin (xanthan) or plant (carrageenan) or fruit origin (pectin); animal connective tissue polysaccharides and proteins, and combinations thereof, such as hyaluronan, proteoglycans and chemically modified animal derived polysaccharides such as hylan; and interactive combinations of these materials which can be associated, bonded and adhered in specific combinations.

In carrying out the process of the invention for producing the new materials from the starting biopolymers, it is preferred that the biopolymer be in its original solid state, i.e., dry, in an atmosphere comprising a mediating agent, preferably a low molecular weight unsaturated alkenic or alkynic gas such as ethylene, propylene or acetylene, preferably acetylene. Before introducing the mediating gas to the reaction site, the site must be flushed to remove therefrom any active, oxygen containing atmosphere. All the mediating gas is removed after completion of the process and therefore, the resulting new materials do not contain any of the mediating gas.

The biopolymeric system (or the finished or partially finished product made therefrom) from which the active atmosphere has been removed is then saturated with the mediating gas at atmospheric pressure and exposed to a source of ionizing radiation which may be either a radioactive isotope such as ^{60}Co (γ-rays) or radiation generated by a high

energy (250 KeV to 10 MeV) electron accelerator or X-rays generated by the accelerator or any other suitable device.

The minimum absorbed radiation dose may vary from 1 kGy to 50 kGy, depending on the structure of the biopolymer, whether branched or long-chain nature of the product desired, whether of increased molecular weight to form a readily water soluble product or to form either a gel or a membrane product. As a general guide, highly branched polysaccharide structures can produce a 4-fold increase in molecular weight with doses up to 10kGy and gels with doses up to 50kGy, whereas straight chain structures can yield a similar change with doses as low as 1-3 kGy. Proteins require doses up to 25kGy to achieve a similar result. Blends and combined adhesive systems require careful dose selection according to the composition of the systems

Following the irradiation step in the presence of the gaseous mediating agent, and in order to remove any activated species produced by the radiation system, the resulting biopolymer system or new material is subjected to heat treatment (annealing) in the absence of oxygen at elevated temperatures ranging from 40°C to 120° C depending on the heat stability of the biopolymer system which is being modified. This annealing step may ideally be carried out in the presence of the unsaturated gaseous atmosphere or, alternatively, in the presence of an inert gas such as nitrogen or helium, or in a vacuum oven. The former can increase the amount of new product formation, and the latter provides a suitable mechanism for termination of the process.

Following the annealing step, any residual gaseous mediating agent is removed from the modified biopolymeric system by aerating the system, and if necessary, the application of a vacuum process to the treated polymer. This will depend on the retention ability of the material for the gas which depends on the porosity of the solid system.

.The resulting new biopolymers obtained by the above-described treatment are characterized by changes in the following parameters when comparing the starting material with the new biopolymers:

    a) molecular weight can be increased 4-5 fold;

    b) increased water binding usually parallels that of the increase in molecular weight;

    c) emulsion with droplet sizes of the order 1 μm can be achieved using reduced concentrations of the biopolymers;

    d) a range of changes in viscosity and viscoelasticity of up to 1000 fold; and

e) there is observed formation of hydrogels having particle sizes from 150-2000 µm.

The molecular weight of the starting biopolymer can be increased in a controlled manner to provide a new generation of products with enhanced properties without losing the basic functionalities of the parent biopolymer. The increased molecular parameters allow greater water binding, improved physical functionalities, such as lower emulsion droplet formation, new binding capabilities and functions to other polymers, whether charged or uncharged, and better fabrication qualities for drug and small ion release.

Water soluble products of increased or decreased viscosity and/or viscoelasticity can be produced. Thus, new food, industrial and medical products can be produced.

Of singular significance to this invention is the fact that no significant or identifiable chemical changes are introduced into the structure of the biopolymer as a result of the process. Thus, any new product made according to the invention will be used in practice in substantially the same way as the parent biopolymer.

Hydrophilic gels (hydrogels) can be produced of a defined particle size and specified micro-mechanical properties.

The products are as biocompatible as are the parent biopolymers.

A wide range of new products can be obtained by changes in the process parameters and these are an integral part of the spirit and purpose of the present invention, and are included within the scope of the claims made herein. The process does not yield a single new product, or even a series of new products within a particular type of biopolymer, but rather, it offers the opportunity of producing a family of new products, each of which may be tailor made for specific applications.

Brief Description of Drawings

The drawings in this application comprise Figures 1 through 22 c, a total of 41 figures on 25 sheets. Each of the figures listed and described below are graphs and are referred to in one or more of the Examples in the section below.

Figure 1   Differential molar mass distribution of control and irradiated *Acacia Senegal*
                 (spray dried)

GSDA-C	control	GSD2-3K	2.3kGy
GSD3-8K	3.8kGy	GSD6-1K	6.lkGy
GSD10-5K	10.5kGy	GSD13-9K	13.9kGy
GSD25A2	24.8kGy	GSD50A 1	49.8kGy

Figure 2   Enhanced production of hydrogels under varying processing conditions.

Figure 3   A comparison of *Acacia Seyal* protein content when subjected to radiation
                 processing; monitored by ultraviolet absorption at 214nm.

TO-7A1	0.7kGy	T1-2AI	1.2kGy
TI-5A1	1.5kGy	T3-2Al	3.2kGy
T8-9A1	5.9kGy	T24-8A1	248kGy
T49-8A1	49.8kGy	T-C2	Control

Figure 4a ^{13}C-NMR of *Acacia senegal* (unirradiated).

Figure 4b ^{13}C-NMR of *Acacia senegal* (irradiated for 6.lkGy)

Figure 5   Shear viscosity profile as a function of shear rates of 33.3% (w/w) control and
                 irradiated *Acacia Senegal* (spray dried). (●) control; (O) 2.3kGy; (▲) 2.8kGy;
                 (△)6.1kGy; (■) 10.5kGy; (□) 13.9kGy.

Figure 6   A plot of (a) storage modulus and (b) loss modulus as a function of frequency
                 of 33.3% (w/w) control and irradiated *Acacia Senegal* (spray dried). (▲)
                 control;   (□) 2.3kGy; (●) 2.8kGy; (x) 3.8kGy; (◇) 6.1kGy; (■) 10.5kGy;
                 (□) 13.9kGy.

Figure 7a   Differential molar mass distribution of control and irradiated Dextran.

Dextran	Control	D0-7A1	0.7kGy
D1-2A1	1.2kGy	D1-5K1	1.5kGy
D1-7K	1.7kGy	D2-3kGy	2.3kGy

D3-8K	3.8kGy	D5-9A1	5.9kGy
D16-2A1	16.2kGy	D24-8A1	24.8kGy
D49-8A1	49.8kGy		

Figure 7b   Storage modules (G') plotted as a function of frequency of 6.25% (w/v) Dextran.

(O) control; (O) 1.5kGy; (+) 1.7kGy; (▲) 2.3kGy; (■) 2.8kGy; (□) 3.8kGy; (▲) 16.2kGy; (x) 24.8kGy.

Figure 8a   Differential molar mass distribution of control and irradiated CMC.

CMC-C2	Control	C1-5K	1.5kGy
C1-7K	1.7kGy	C2-3K	2.3kGy
C2-8K	2.8kGy	C3-8K	8.3kGy

Figure 8b   Shear viscosity profile as a function of shear rates of control and irradiated 2% in H_2O of CMC. (O) control; (▲) 1.5kGy; (□) 2.8kGy.

Figure 8c   Storage modulus (G'); plotted as a function of frequency for 2% in H_2O of control and irradiated CMC (O) control; (▲) 1.5kGy; (□) 2.8kGy.

Figure 9a   Differential molar mass distribution of control and irradiated pullulan

P-C2	Control	P1-2AI	1.2kGy
P1-5K	1.5kGy	P1-7K	1.7kGy
P2-3K	2.3kGy	P2-8K	2.8kGy
P3-8K	3.8kGy	P5-9A1	5.9kGy
P24-8A1	24.8kGy	P49-8A1	49.8kGy

Figure 9b   Storage modulus (G'); plotted as a function of frequency of control and irradiated 20% pullulan. (O) control; (●) 2.3kGy; (■) 2.8kGy.

Figure 10a   Shear viscosity as a function of shear rates for 0.5% in H_2O of irradiated hyaluronan compared with control.

Figure 10b   Loss modulus (G") plotted as a function of frequency for 0.4% in H_2O of irradiated hyaluronan compared with the control.

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Figure 10c   Infra red scans of irradiated hylan fibers.

Figure 11a   Storage modulus (G') plotted as a function of frequency of 1% in H_2O of
                control and irradiated xanthan. The solution was heated for 20 min. at 85°C
                and the measurements were performed at 25°C. (O) control; (●) 0.7kGy,

Figure 11b   Loss modulus (G") plotted as a function of frequency of 1% in H_2O of control
                and irradiated xanthan. The solution was heated for 20 min. at 85°C and the
                measurements were performed at 25°C. (O) control; (●) 0.7kGy.

Figure 11c   Dynamic viscosity (η') plotted as a function of frequency of 1% in H_2O of
                control and irradiated xanthan. The solution was heated for 20 min. at 85°C
                and the measurements were performed at 25°C. (O) control; (●) 0.7kGy.

Figure 12   Shear viscosity profile as a function of shear rate of 1% in H_2O of control and
                irradiated kappa-carrageenan. The solution was heated for 20 min. at 85°C
                and the measurements were performed at 25°. (O) control; (●) 16.2kGy; (▲)
                24.8kGy.

Figure 13   Storage modulus (G") plotted as a function of frequency of 3.6% (w/v) in H_2O
                of control and irradiated orange pectin. (▲) control; (●) 3kGy.

Figure 14   (a) Storage modulus (G'), (b) loss modulus (G") and (c) dynamic viscosity
                plotted as a function of frequency of 10% (w/v) in H_2O of control and
                irradiated gelatin. Samples were heated for 15 min. at 50°C and the
                measurements were carried out on cooled samples at 25°C. (O) control; (●)
                3kGy; (▲) 10kGy.

Figure 15   Storage modulus (G'); plotted as a function of frequency of 4% (w/v) in H_2O
                of control and irradiated mix containing 90% orange pectin + 10% CMC. (O)
                control; (●) 3.3kGy; (▲) 10.4kGy.

Figure 16   Storage modulus (G'), plotted as a function of frequency of 4% (w/v) in H_2O
                of control and irradiated mix containing 90% orange pectin + 10% dextran.
                (O) control; (●) 3.3kGy; (,29) 10.4kGy.

Figure 17   Storage modulus (G'), plotted as a function of frequency of 4% (w/v) in H_2O

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of control and irradiated mix containing 50% dextran + 50% CMC. (O) control;   (●) 3.3kGy; (▲) 10.4kGy.

Figure 18   Dynamic viscosity plotted as a function of frequency of 4% (w/v) in H_2O of control and irradiated mix containing 90% polyvinyl pyrrolidone (PVP) + 10% Dextran.   (O) control; (□) 10 4kGy processed together; (■) 10.4kGy processed separately.

Figure 19   (a) Storage modulus (G'); (b) Loss modulus (G"); and (c) Dynamic viscosity (η') plotted as a function of frequency of 4% (w/v) in H_2O of control and irradiated mix containing 50% polyvinyl pyrrolidone (PVP) + 50% arabinogalactan protein (Gum Arabic). (▲) control; (●) 3.1kGy processed together; (△) 3.1kGy processed separately; (■) 10.4kGy processed together; (□) 10.4kGy processed separately.

Figure 20   (a) Storage modulus (G'); (b) loss modulus (G"); (c) dynamic viscosity (η) plotted as a function of frequency of 25% (w/v) in H_2O of control and irradiated mix containing 10% carboxymethyl cellulose (CMC) + 90% arabinogalactan protein (Gum Arabic).
(O) control; (●) 3.1kGy processed together; (△) 3.1kGy processed separately.

Figure 21   GPC chromatogram of control and irradiated mix containing 10% carboxymethyl cellulose (CMC) + 90% arabinogalactan protein (Gum Arabic).

C-M6 Control   3-M6 3.1kGy   10-M6 10.4kGy

Figure 22   (a) Storage modulus (G'); (b) loss modulus (G"); (c) dynamic viscosity (η) plotted as a function of frequency of 4% (w/v) in H_2O of control and irradiated mix containing 90% orange pectin + 10% dextran.
(▲) control; (●) 3.1kGy processed together; (O) 3.1kGy processed separately; (■) 10.4kGy processed together; (□) 10.4kGy processed separately.

Best Mode for Carrying Out the Invention

The invention will now be described in greater detail in the following examples which are not intended to limit the scope of the invention but are merely explanatory thereof.

Example 1

Arabinogalactan Proteins

The structures, properties and functionality of the gum exudates *Acacia Senegal* and *Acacia Seyal* have previously been elucidated by us and these species are included within the scope of this example and the invention. They are also included within the specifications of the material commercially known as Gum Arabic of Commerce.

These materials are widely used in industry as adhesives, stabilizers, emulsifiers, flavor encapsulators, materials for preventing sugar crystallization (in confectionery), stabilizing printing ink, etc. This material is a globular cross-linked polysaccharide made up of arabinose, galactose, rhamnose and uronic acid joined together in polysaccharide units of ca. 400,000 molecular weight (MW), with each of the globular polysaccharide units joined together like beads on a string to a protein chain. The protein composition is about 3%. The arabinogalactan proteins are not discrete molecular entities, since on hydrophobic or size exclusion fractionation, two main components can be differentiated with MW ca 1×10^6 and 4 -5 $\times 10^5$.

Characterizing properties associated with the new products produced

A Controlled Increase in Molecular Weight

The process allows a controlled increased molecular weight of arabinogalactans. The starting material is first fractionated using gel permeation chromatography (GPC) column coupled to a multi-angle laser light scattering detector (MALLS), a concentration detector (Refractive index – RI) and UV absorbance detector at 214 nm. The system allows the molecular weight distribution of the gum to be measured using multi-angle laser light scattering, and thus any changes in the overall structure can be monitored using the three detectors. Typical changes in molecular weight distribution is shown in Figure 1 and the quantitative results are given in Table 1 for a representative spray dried *Acacia Senegal* gum. To illustrate that the spray drying processing has no influence on the generality of the changes observed, we have also carried out exactly the same experiments using unprocessed (lump) *Acacia Senegal* gum and the results are shown in Table 2, which are directly comparable A 4-fold -soluble. There is a corresponding increase in the root mean square radius of the processed gum.

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Table 1.   Acacia senegal: an arabinogalactan protein (spray dried). % M_w means the mass recovered after injection. Rg is the RMS-radius and P is the polydispersity (P = M_w/M_n).

File name	M_{wt} processed as one peak	% M_{wt}	R_g nm	P	M_{wt} processed as two peaks	% M_{wt}	R_g nm	P	Comments
GSDSA-C	$8.42 \times 10^6 \pm 0.56$	98	-	2.28	$2.41 \times 10^6 \pm 0.15$ $4.18 \times 10^5 \pm 0.25$	20.9 77.4	13.3 -	1.40 1.37	Control
GSD2-3K	$2.32 \times 10^6 \pm 0.21$	96.8	8.3	4.78	$7.20 \times 10^6 \pm 0.67$ $4.76 \times 10^5 \pm 0.43$	26.5 70.6	21.2 -	2.38 1.28	2.3KGy
GSD2-8K	$3.44 \times 10^6 \pm 0.30$	92.1	27.8	5.85	$1.07 \times 10^6 \pm 0.09$ $5.71 \times 10^5 \pm 0.11$	26.1 66.1	28.7 27.4	2.37 1.30	2.8KGy
GSD3-8K	$3.69 \times 10^6 \pm 0.33$	83.7	29.5	6.09	$1.06 \times 10^6 \pm 0.09$ $5.52 \times 10^5 \pm 0.48$	26.0 57.9	30.1 29.3	2.59 1.25	3.8KGy
GSD6-1K	$3.12 \times 10^6 \pm 0.26$	63.1	31.9	6.0	$1.12 \times 10^6 \pm 0.09$ $5.30 \times 10^5 \pm 0.39$	15.2 47.9	34.4 31.0	2.63 1.30	6.1KGy
GSD10-5K	$1.81 \times 10^6 \pm 0.16$	47.6	29.3	5.65	$7.93 \times 10^5 \pm 0.72$ $3.65 \times 10^5 \pm 0.32$	9.1 37.8	32.6 27.8	2.51 1.37	10.5KGy
GSD13-9K	$1.54 \times 10^6 \pm 0.17$	37.5	26.3	5.70	$7.11 \times 10^5 \pm 0.79$ $5.30 \times 10^5 \pm 0.39$	6.8 30.0	31.0 23.0	2.50 1.39	13.9KGy
GSD25A	$7.98 \times 10^5 \pm 0.71$	18.3	-	4.48	$7.32 \times 10^5 \pm 0.55$ $2.69 \times 10^5 \pm 0.19$	9.1 16.5	32.6 38.2	2.51 1.74	24.8KGy
GSD49-8A	$3.27 \times 10^5 \pm 0.53$	12.4	-	2.18	$2.48 \times 10^5 \pm 0.56$ $2.01 \times 10^5 \pm 0.32$	0.33 12.2	27.6 -	1.5 1.39	49.8KGy

Table 2. Acacia Senegal gum (unprocessed/lump gum). % M_w means the mass recovered after injection. Rg is the RMS-radius and P is the polydispersity (P=M_w/M_n).

File name	M_{wt} processed as one peak	% M_w	R_g nm	P	M_{wt} processed as two peaks	% M_w	R_g nm	P	Comments
HASHAB	$4.92 \times 10^5 \pm 0.25$	104	-	1.73	$1.64 \times 10^6 \pm 0.08$ $2.96 \times 10^5 \pm 0.15$	15.1 89.5	10 -	1.26 1.18	Control
HAS0-7A1	$5.71 \times 10^5 \pm 0.14$	107	24.8	2.10	$2.19 \times 10^6 \pm 0.05$ $2.92 \times 10^5 \pm 0.08$	15.7 91.9	28.9 24.0	1.43 1.23	0.7KGy
HAS1-2A1	$7.88 \times 10^5 \pm 0.19$	110	17.5	2.67	$3.13 \times 10^6 \pm 0.09$ $3.18 \times 10^5 \pm 0.05$	18.6 93.1	30.8 13.2	1.63 1.26	1.2KGy
HSA2-3K	$6.94 \times 10^6 \pm 0.59$	88.9	-	3.23	$3.46 \times 10^6 \pm 0.31$ $2.49 \times 10^5 \pm 0.19$	15.7 97.7	21.5 -	1.95 1.31	2.3KGy
HSA2-8K	$1.04 \times 10^6 \pm 0.08$	110	-	3.97	$4.45 \times 10^6 \pm 0.35$ $2.77 \times 10^6 \pm 0.21$	20.1 90.1	23.3 -	2.14 1.27	2.8KGy
HSA3-8K	$9.99 \times 10^6 \pm 0.77$	113	-	3.67	$4.76 \times 10^6 \pm 0.37$ $2.96 \times 10^6 \pm 0.21$	17.8 96.0	25.9 -	2.17 1.26	3.8KGy
HAS5-9A	$1.12 \times 10^6 \pm 0.27$	108	29.1	3.54	$4.25 \times 10^6 \pm 0.09$ $3.40 \times 10^5 \pm 0.09$	22.0 87.3	35.5 27.3	1.82 1.30	5.9KGy
HSA6-1K	$1.31 \times 10^6 \pm 0.12$	108	-	5.09	$6.30 \times 10^6 \pm 0.61$ $2.83 \times 10^5 \pm 0.26$	18.6 90.2	26.4 -	2.56 1.29	6.1KGy
HSA10-5K	$1.37 \times 10^6 \pm 0.09$	91.9	23.0	4.55	$5.80 \times 10^6 \pm 0.40$ $3.18 \times 10^5 \pm 0.21$	17.7 74.4	32.2 20.3	2.43 1.27	10.5KGy
HSA13-9K	$1.30 \times 10^6 \pm 0.14$	76.8	-	4.85	$6.39 \times 10^6 \pm 0.70$ $2.97 \times 10^5 \pm 0.31$	12.6 64.2	19.8 -	2.43 1.30	13.9KGy
HAS16-2A	$1.38 \times 10^6 \pm 0.03$	85.6	28.6	4.53	$5.79 \times 10^6 \pm 0.14$ $3.50 \times 10^5 \pm 0.08$	16.1 69.5	35.9 26.5	2.06 1.38	16.2KGy
HAS24-8A	$1.27 \times 10^6 \pm 0.37$	70	27.7	4.27	$5.83 \times 10^6 \pm 0.07$ $3.27 \times 10^5 \pm 0.06$	12.0 57.7	35.4 25.8	2.12 1.29	24.8KGy
HAS49-8A	$1.25 \times 10^6 \pm 0.03$	75	34.2	3.97	$5.14 \times 10^6 \pm 0.15$ $3.45 \times 10^5 \pm 0.09$	14.3 60.8	35.8 33.8	2.06 1.30	49.8KGy

2   Conversion into Hydrogel Form (of varying dimensions)

By controlled delivery of the dose of radiation, the high molecular weight acacia gum can be converted into a hydrogel according to the following pattern:

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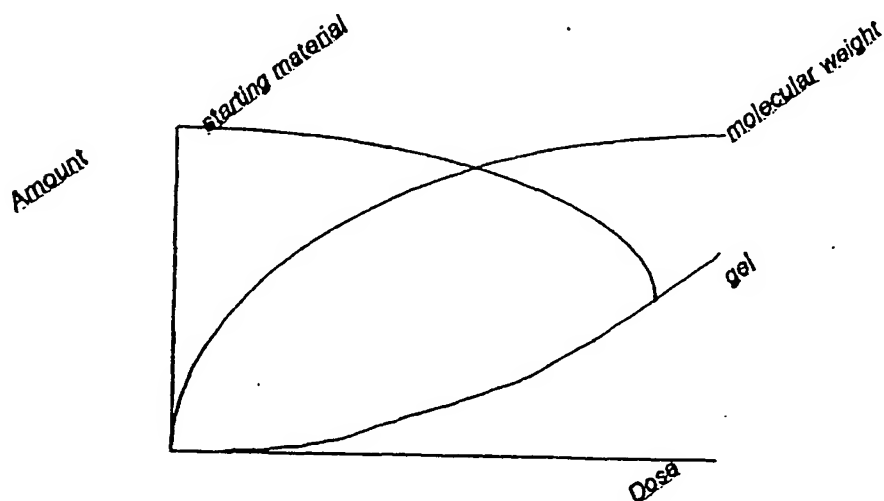
Tables 1 and 2 also illustrate how it is possible to progressively convert any amount or proportion of the soluble arabinoglactan protein into hydrogel. Moreover the particle size of the hydrogel can be varied according to need. Coulter counter distributions show, for example, this variation from a mean of 160 μm upwards into solidified hydrogel systems at 2000μm and beyond (Figure 2). The pattern then is of a progressive increase in MW, with a portion even reaching 100 x10⁶ , linking up at this stage to give gel particles which are quite visible. These particles are not even able to enter the fractionating column, and consume the highest MW material, which accounts for the levelling off of the soluble gum after 6.1 Kgy.

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Increasing the Proportion of the Higher Molecular Weight Emulsification Component

The high molecular weight arabinogalactan protein (AGP) component of Acacia gums is responsible for the emulsification effectiveness of oil in water emulsion. The higher proportion in *Acacia Senegal* compared with *Acacia Seyal* makes this gum more valuable, and as such commands a higher price. The AGP coats the oil droplets and prevents them from re-associating. Therefore, such an emulsion is stable for months and even years. To increase the amount of this component would in itself give added value to the gum. We are able to demonstrate that by applying the radiation processing

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method to *Acacia Seyal* the proportion of the higher molecular weight component (AGP) can be increased. This increase is reflected by the molecular weight distribution and the RMS-radius and so gives a new material with properties more akin to A. senegal (Table 3).

Table 3. *Acacia Seyal* (Talha). % M_w means the mass recovered after injection. Rg is the RMS-radius and P is the polydispersity (P = M_w/M_n).

File name	M_{wt} processed as one peak	% M_{wt}	R_g nm	P	M_{wt} processed as two peaks	% M_{wt}	R_g nm	P	Comments
T-C1	$1.15 \times 10^6 \pm 0.02$	106	20.6	1.98	$2.98 \times 10^6 \pm 0.05$ / $5.58 \times 10^5 \pm 0.20$	26.58 / 2.3	26.11 / 8.5	1.37 / 1.28	Control
T0-7A1	$1.65 \times 10^6 \pm 0.33$	103	25.1	2.89	$4.45 \times 10^6 \pm 0.09$ / $5.44 \times 10^5 \pm 0.09$	29.27 / 4.0	31.12 / 2.0	1.65 / 1.27	0.7kGy
T1-2A1	$1.67 \times 10^6 \pm 0.02$	101	23.2	2.32	$3.81 \times 10^6 \pm 0.06$ / $6.09 \times 10^5 \pm 0.07$	33.56 / 8.2	27.8 / 20.8	1.54 / 1.14	1.2kGy
T1-5A1	$1.78 \times 10^6 \pm 0.39$	108	21.5	2.51	$4.31 \times 10^6 \pm 0.07$ / $5.72 \times 10^5 \pm 0.07$	28.17 / 1.9	36.91 / 7.2	1.63 / 1.13	1.5kGy
T3-2A1	$3.17 \times 10^6 \pm 0.06$	105	30.4	3.91	$7.64 \times 10^6 \pm 0.16$ / $6.15 \times 10^5 \pm 0.09$	38.16 / 8.1	35.4 / 27.2	2.18 / 1.14	3.2kGy
T5-9A1	$3.33 \times 10^6 \pm 0.83$	99.1	35.1	4.31	$8.61 \times 10^6 \pm 0.22$ / $6.49 \times 10^5 \pm 0.12$	33.46 / 6.0	38.43 / 3.3	2.26 / 1.17	5.9kGy
T24-8A1	$3.07 \times 10^6 \pm 0.06$	78.5	33.5	3.74	$7.05 \times 10^6 \pm 0.14$ / $6.31 \times 10^5 \pm 0.12$	29.64 / 8.0	35.9 / 32.1	2.09 / 1.14	24.8kGy
T49-8A1	$3.05 \times 10^6 \pm 0.07$	72.4	35.0	4.17	$7.33 \times 10^6 \pm 0.17$ / $5.70 \times 10^5 \pm 0.10$	26.64 / 5.4	37.33 / 3.6	2.23 / 1.14	49.8kGy

Table 3 illustrates how the high molecular weight component can be increased in molecular weight from 3 to 8 million, and its amount increased from 26% to 38%. Using hydrophobic interaction chromatography (HIC) the individual components were fractionated. The results confirm the increased proportion of the AGP component reflected by the increased amount of protein by UV monitoring at 214 nm as shown in Fig 3.

Improved Emulsification Performance

The modified acacia gum samples were evaluated. The results of the evaluation of emulsification effectiveness showed:

When *Acacia Senegal* was converted into a new product with increased molecular weight, it proved to be a better emulsifier than the original unprocessed control

When *Acacia Seyal*, the least effective emulsifier compared with *Acacia Senegal* was processed to increase its molecular weight and to give the same amount of high molecular weight component as *Acacia Senegal*, its performance was as good. Thus in effect, the *Acacia Seyal* had been converted at least with respect to its emulsification behavior, to the more valuable *Acacia Senegal*.

No Introduction of New Chemical Groups

The doses of radiation are relatively low in relation to those which would induce significant chemical change when the same material is irradiated in the absence of the mediating gas. Chemical analyses have shown that there is no significant structural change introduced by the process as shown in Table 4.

Table 4.   Physicochemical and Chemical Properties of
Original and Irradiated Gum (25kGy 10Mev electrons
volt)

	Control	Irradiated
% moisture	12.5	12.4
Specific rotation	+52	+52
Limiting viscosity (cc/g)	12	16
% sugars		
Rhamnose	5	4
Arabinose	45	46
Galactose	34	34
Glucuronic acid	15	15
Nitrogen	0.15	0.15

For precise evaluation ^{13}C-NMR Nuclear magnetic resonance spectra of the unprocessed and processed gum for which maximum molecular weight change had been found were compared. No difference was found between the processed and unprocessed gum (Figures 4a and 4b). Thus no new chemical groups had been introduced as a result of the processing.

Controlled Increase in Viscosity and Viscoelasticity

The changes in shear viscosity with shear rate reflect the molecular weight changes which were found. Initially the gum is a compact globular system, with no significant effect of shear. At this stage the polysaccharide acts as a set of small compact balls, with no shear thinning. As the dose of radiation used for processing is increased, an entangled network is produced, typical of longer entangled molecules when shear thinning can be observed (Figure 5). There is at least 1000-fold increase in viscosity at zero shear after a dose of 13kGy.

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Oscillation measurements also confirmed the above observations (Figures 6a and

6b). Here, the storage modulus (G' - Fig 6a) and the loss modulus (G" - Fig 6b) are plotted as a function of frequency. The dynamic network build up, is again, reflected by the change from dilute solution response (that is G" > G') to concentrated solution (G' > G") with a cross-over frequency shifted to lower frequency with increasing Mw) and finally to strong gel behavior where G' and G" are independent of frequency. The dynamic viscosity is similar to shear viscosity profile given in Figure 5, with shear thinning developing, as a result of building a network structure capable of entanglements, with increasing dose. Thus the viscosity can be increased as required and when necessary the system can be converted into a hydrogel, which is a highly viscoelastic state.

Improved   Interaction with Water   (Enhanced Water Binding Properties)

As a result of increasing its molecular dimensions, it is possible to control the water binding properties of the arabinoglactan protein. This was demonstrated using pulsed nuclear magnetic resonance spectrometry.

The spin-spin relaxation times (T_2) for the processed and unprocessed *Acacia Senegal* and *Acacia Seyal* aqueous solutions are shown in Table 5. Measurements were carried out using a 25 MHz Pulse NMR at 30°C. Completely soluble *Acacia Seyal* sample were selected, and here only one kind of T_2 is evident. Since T_2 of bulk water varies from 2 to 4 sec, and the values found for the *Acacia Seyal* samples are 1 sec or less, it is evident that the molecular motion of the water is restricted by the acacia gum. In other words the acacia samples bind water. Our studies using differential scanning calorimetry have previously demonstrated the effectiveness of the acacia gum in binding water. See "Hydration characteristics of the gum exudate

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from *Acacia Senegal*, G. O. Phillips, et al, Food Hydrocolloids, Vol 10 (1), 1996, pp 11-19.

As shown in Table 5, the value of T_2 decreases with molecular weight. This shows that the amount of bound water increases with molecular weight.

The spray dried *Acacia Senegal* samples showed two kinds of T_2 values, since the selected gums have a gel component. The same trend however is shown for the increased water binding with increased molecular weight for the free water component. The reverse is true for the gel component. With increased molecular weight, the hydrogel structure is more loose and the extent of the water immobilization decreases with molecular weight.

The T_2 of the dry samples was also measured and is listed in Table 6. Both types of samples show two kinds of T_2. T_{2S} relates to the relaxation time for the proton of the OH groups in the gum and T_{2L} is the relaxation time for the absorbed water. Again there is an effect of molecular weight to decrease relaxation time, which fits in with the aqueous solution observations and those carried out on the "dry" samples.

Table 5. Spin-spin relaxation time (T_2) for Gum Arabic aqueous solutions. Measurements were carried out using a pulse NMR at 30°C.

Sample	Conc.	T_{2s}	T_{2L}	Treatment
Seyal (1.2)	10.0	-	1000.00 (100%)	Control
Seyal (3.3)	10.0	-	980.34 (100%)	5.9kGy
Seyal (3.0)	10.0	-	908.18 (100%)	49.8kGy
Senegal (0.84)	10.0	55.79 (5.6%)	374.19 (94.4%)	Control
Senegal (3.1)	9.91	78.13 (7.9%)	341.91 (92.1%)	6.1kGy
Senegal (3.3)	10.0	112.54 (57.2%)	368.20 (42.8%)	49.8kGy

Table 6. Spin-spin relaxation time (T_2) for dry Gum Arabic samples. Measurements were carried out using a pulse NMR at 30°C.

Sample MW x 10^6	T_{2s} (μsec)	(%)	T_{2L} (μsec)	(%)	Treatment
Seyal (1.2)	15.58	(74.9%)	242.11	(25.1%)	Control
Seyal (3.3)	15.45	(77.9%)	207.82	(22.1%)	5.9kGy
Seyal (3.0)	15.64	(76.9%)	202.43	(23.1%)	49.8kGy
Senegal (0.84)	16.51	(75.8%)	309.93	(24.2%)	Control
Senegal (3.1)	15.95	(77.3%)	284.97	(22.7%)	6.1kGy
Senegal (3.3)	15.95	(78.4%)	265.98	(21.6%)	49.8kGy

Example 2

Natural (unsubstituted) and chemically modified Polysaccharides

The identical type of modification can be achieved with the widest range of polysaccharides available. Here polysaccharides typical of the various groups will be described. The results will be summarized, but the type of information already described is available and forms part of this claim.

The process modification herein described can be achieved with the widest range of polysaccharide systems available. It is not possible to illustrate this with every single polysaccharide and those which have been chemically modified. Nevertheless, we have selected representative samples from the various groups and families to show that similar changes can be produced over the whole range of such materials and that the behavior is universal. In this section, we provide illustrative examples commensurate in scope with the product claims set forth below.

DEXTRAN - a bacterial branched chain polysaccharide.

As is known, the basic skeleton of dextran consists of (1-6) linked D-glucose units with

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side chains attached to 0 - 3 of the backbone chain units. The degree of branching has been determined to be 5%. There is also some indication of a small proportion of α-D(1-3) branches. However, many aspects of the fine structure remain to be resolved. Dextran is used as blood-plasma substitutes and for this purpose needs to have a variety of molecular weights. They are also used as standards for the measurement of molecular weights. For this reason it would be advantageous to have a method which would precisely tailor the molecular weight to particular values.

The dextran selected for the example is of high average MW (1.44×10^6), with two peaks observable in the chromatogram. The weight average molecular weight of each peak was determined and found to be 2.34×10^6 and 2.05×10^5. After a dose of 1.2kGy radiation, there was an increase in MW of the average (3.04×10^6) and highest MW component (4.58×10^6). The molar mass distributions illustrate the overall increase in MW distribution. (Fig. 7a) The gel formation starts after a dose of 1.2KGy as reflected by the decrease in the mass recovery (Table 7). Again, the gel formation increases with the irradiation dose. As the dose is increased, the MW levels off and then decreases as gel is formed by cross-linking of the high MW soluble polysaccharide. After a dose of 49.8kGy, approximately 83% of the original material will not pass through a 1μm filter and was used to prepare the gel, which is visually observable. The root mean square radii (RMS) confirm the increases in MW of the disperse polysaccharide system, increasing from the initial value of 26.8 nm to ~43 nm at the highest MW attained. Polydispersity (P) similarly increases showing the broadening of the molecular weight distribution at the higher molecular weight end of the chromatogram (from 4 to 5). Details of the quantitative changes are given in Table 7.

Table 7. Changes in molecular weight and RMS-radius of processed Dextran. % M_w means the mass recovered after injection. Rg is the RMS radius and P is the polydispersity ($P = M_w/M_n$).

Filename	M_{wt} processed as one peak	% M_{wt}	R_g nm	P	M_{wt} processed as two peaks	% M_{wt}	R_g nm	P	Dose
Dextran	$1.44 \times 10^6 \pm 0.10$	105	16.8	3.98	$2.34 \times 10^6 \pm 0.16$	67.3	21.2	1.84	Control
					$2.05 \times 10^5 \pm 0.25$	43.4	5.5	1.13	
D0-7A1	$2.38 \times 10^6 \pm 0.49$	100	37.3	3.88	$3.58 \times 10^6 \pm 0.13$	64.4	39.5	2.02	0.7KGy
					$3.08 \times 10^5 \pm 0.07$	36.8	32.8	1.07	
D1-2A1	$3.04 \times 10^6 \pm 0.09$	103	43.3	4.3	$4.58 \times 10^6 \pm 0.13$	65.7	43.9	2.41	1.5KGy
					$3.50 \times 10^5 \pm 0.08$	38.05	43.9	1.05	
D1-5K	$2.16 \times 10^6 \pm 0.09$	92.2	35.7	5.35	$3.65 \times 10^6 \pm 0.15$	52.0	37.7	2.70	1.5KGy
					$2.30 \times 10^5 \pm 0.10$	41.2	33.0	1.08	
D1-7K	$1.96 \times 10^6 \pm 0.08$	93.0	34.5	5.07	$3.56 \times 10^6 \pm 0.14$	48.2	36.1	2.61	1.7KGy
					$2.42 \times 10^5 \pm 0.09$	45.2	32.8	1.10	
D2-3K	$1.62 \times 10^6 \pm 0.07$	85.0	30.6	5.24	$3.11 \times 10^6 \pm 0.14$	41.0	34.6	2.45	2.3KGy
					$2.10 \times 10^5 \pm 0.11$	44.0	26.2	1.16	
D2-8K	$1.21 \times 10^6 \pm 0.05$	71	27.1	5.23	$2.51 \times 10^6 \pm 0.11$	31.3	32.4	2.38	2.8KGy
					$1.85 \times 10^5 \pm 0.09$	40.0	22.5	1.31	
D3-8K	$9.69 \times 10^5 \pm 0.08$	66	24.3	5.10	$2.30 \times 10^6 \pm 0.09$	24.6	32.1	2.23	3.8KGy
					$1.74 \times 10^5 \pm 0.07$	42.0	18.0	1.36	
D5-9A1	$9.30 \times 10^5 \pm 0.41$	43	36.9	4.17	$2.66 \times 10^6 \pm 0.12$	11.6	38.6	2.65	5.9KGy
					$2.08 \times 10^5 \pm 0.09$	31.5	36.0	1.20	
D16.2A1	$3.54 \times 10^5 \pm 0.24$	27	34.1	4.39	$1.99 \times 10^6 \pm 0.15$	3.1	36.5	2.68	16.2KGy
					$1.20 \times 10^5 \pm 0.05$	23.7	32.7	1.67	
D24-8A1	$1.75 \times 10^5 \pm 0.18$	22	-	4.97	$1.15 \times 10^6 \pm 0.14$	1.29	38.6		24.8KGy
					$9.80 \times 10^5 \pm 0.90$	20.8	-		
D49-8A1	$1.72 \times 10^5 \pm 0.40$	17	-	4.42					49.8]KGy

Rheologically, the changes in viscosity of the processed dextran are very marked. Initially the shear viscosity profile shows a Newtonian behavior which changes to shear thinning with increasing radiation dose. Significantly even though the overall MW shows a decrease at 24.8kGy, the viscosity is maintained, indicating that the gel-like character of the system is built up prior to gel formation. Oscillation measurements confirmed the above observation where the increase in G' and G" as a function of irradiation dose is shown in Figure 7b where the changes from dilute solution response to concentrated solution and finally to gel like response are clearly demonstrated.

The changes below illustrate schematically the rapid conversion of the soluble material into gel. By careful dose and MW selection, the required system can be tailor-made.
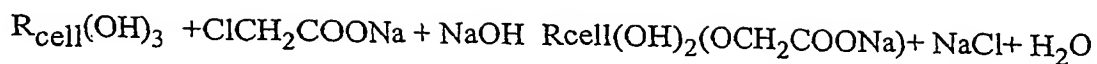
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Here the gel particles show a distribution from 20 – 600 μm.
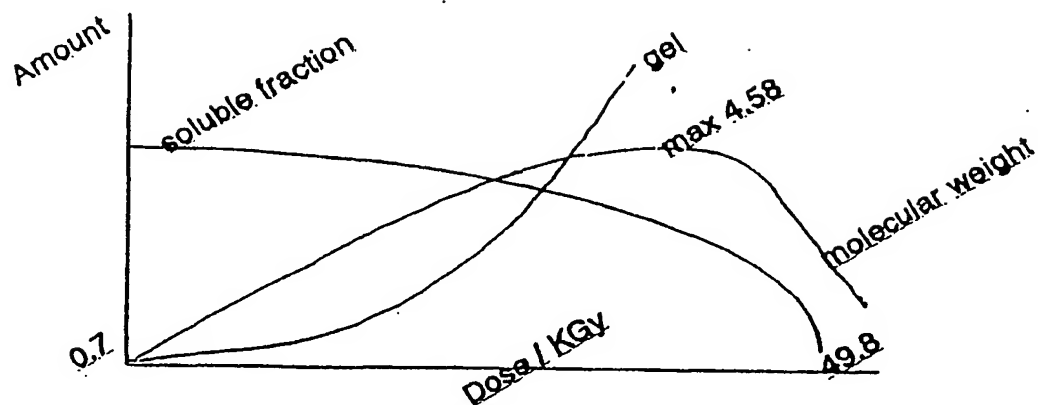
Carboxymethyl cellulose (CMC): a chemically modified polysaccharide.

When cellulose is steeped in sodium hydroxide solution and the alkali cellulose

etherified with sodium monochloracetate, sodium carboxymethyl cellulose is

produced:

$$R_{cell}(OH)_3 + ClCH_2COONa + NaOH \; Rcell(OH)_2(OCH_2COONa) + NaCl + H_2O$$

Because three reactive hydroxyl groups are present on each D-glucose-pyranosyl unit, it is possible to introduce three sodium carboxymethyl groups per unit. Such a product would be described as having a degree of substitution of 3. Commercial CMC generally has an average DS of less than 1.5. The derivatized product, having been derived from cellulose with its β - 1-4 link preserved is linear in character, and was selected as a structure different from the globular and cross-linked polysaccharides previously described.

A summary of the results is given in Table 8. It is clear that the structural changes can again be achieved using the radiation processing, even in this linear structure, which of course would be more susceptible to radiation induced chain scission effects. The initial mean MW of 1.55×10^5, with a dose of 1.5kGy is increased three-fold to 4.44×10^5. Moreover the polydispersity is improved from 2 to 2.8. This is quite apparent in the differential (Figure 8a) molar mass distribution, and the increase in R_g from 36 to 52 (see Table 8).

Table 8. CMC (7M). % M_w means the mass recovered after injection. Rg is the RMS-radius and P is the polydispersity ($P = M_w/M_n$).

File name	M_{wt}	% M_{wt}	R_g nm	P	Mean particles diameter (μm)	Treatment
CMC-C	$1.55 \times 10^5 \pm 0.10$	100	36.4	2.0	None	Control
C1-5K	$4.44 \times 10^5 \pm 0.25$	73	51.9	1.50	215	1.5kGy
C1-7K	$3.94 \times 10^5 \pm 0.17$	82	51.7	1.8	245	1.7kGy
C2-3K	$3.57 \times 10^5 \pm 0.15$	81	47.9	2.23	310	2.3kGy
C2-8K	$3.13 \times 10^5 \pm 0.14$	74	46.8	2.82	434	2.8kGy
C3-8K	$2.71 \times 10^5 \pm 0.12$	64	31.3	2.39	532	3.8kGy

The changes in viscosity (Figure 8b) reflect the MW changes, with the now anticipated shear thinning associated with the higher molecular weight product. Gel is formed at the higher doses and is visible in solution. Gelation of CMC solution can be controlled to give stable gels ranging in consistency from soft pourable to very firm. Oscillation measurements are given in Figures 8c and d for G' and G" respectively. At a frequency of 0.1Hz there is a 10-fold increase in G' and G". The method allows controlled increase in molecular weight and gel formation which are increased linearly with the radiation dose as shown in Table 8.

Samples of the modified CMC were subjected to evaluation of its effectiveness in baking applications. The evaluation was extremely positive as shown in the summary of the report based on Farinograph data of three modified samples:

Table 9. An independent evaluation of processed CMC.

Sample	Water absorption %	Dough stability Min.	Dough softening BU
The Company Standard	62.0	6.8	60
Unprocessed Control	67.6	7.7	65
C1	65.7	15.5	15
C2	62.3	18.3	20
C3	68.4	12.3	30

The following explanation will assist in the interpretation of these parameters:
Standard CMC is a 7 MF which is the starting material.

Dough softening is the change in consistency during mixing. The better the dough maintains its consistency, the better, and consequently a good dough stability (measured in minutes) and a low dough softening (measured in BRABENDER UNITS (an arbitrary unit) is the target.

The dough stability is the number of minutes the dough maintains the same consistency, the dough softening is the change in consistency after a fixed number of minutes (typically 12 minutes)

Water absorption is the amount of water added in order to obtain a fixed consistency – i.e. if a flour has high water absorption, it requires more water to obtain this fixed consistency than a flour with low water absorption. Addition of hydrocolloids can increase the water absorption, but often it results in lower dough stability and higher dough softening - which is logical as the dough contains more water.

Conclusion: As seen from Table 9, the samples provided showed good water absorption properties, excellent dough stability and resistance to dough softening. This means that a very stable dough with excellent mixing properties and good water absorption can be made using our modified CMC samples C1, C2 and C3.

Pullulan : a fungal polysaccharide

Pullulan is a glucan which is extracellularly elaborated by a fungus of the genus *Aureobasidium*. It is a linear glucan consisting of repeating units of maltotriose joined by α-D-(1-6) linkages. Pullulan hydrolyses produced by pullanase yield about 6.6% maltotetraose, indicating that pullulan contains some maltotetraose units.

Pullulan was selected for the example because it is intermediate between the straight chain CMC and the cross-linked dextran and highly branched globular arabinogalactan proteins. On radiation processing the average MW doubles from 3.17×10^5 to 6.81×10^5 and maintains this level to 2.8kGy; and moreover, there is conversion of the original material to form gel after 3.8kGy. After a dose 49.8KGy there is some 30% of the original material converted into gel particles (Table 10).

It is evident that doses and irradiation conditions need to be identified for each system to achieve the optimum results. The initial molecular weight distribution shows two peaks, with one centred on a molar mass of 2.3×10^5 but moves to 3.5×10^5. The second high molecular weight peak, initially at 6×10^5 moves on processing to 1.3×10^6. The molecular weight distribution broadens (Figure 9a) and there is a decrease in the original differential weight fractions as the higher molecular weight material is being formed. Measurements of G' (Fig. 9b) shows the enhancement of the rheological properties in manner expected for high molecular weight materials. The dynamic viscosity increase on processing correlates well with the above mentioned Figure.

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Table 10. Pullulan. % M_w means the mass recovered after injection. Rg is the RMS radius and P is the polydispersity ($P = M_w/M_n$).

File name	M_{wt} processed as one peak	% M_{wt}	R_g nm	P	M_{wt} processed as two peaks	% M_{wt}	R_g nm	P	Comments
Pullulan	$3.17 \times 10^5 \pm 0.16$	118	30.1	2.74					
P1-2A1	$4.84 \times 10^5 \pm 0.18$	105	32	2.27	$1.13 \times 10^6 \pm 0.03$ / $1.97 \times 10^5 \pm 0.10$	32.3 / 73.9	37.7 / 29.5	1.43 / 1.21	1.2KGy
P1-5K	$6.35 \times 10^5 \pm 0.35$	110	25.1	3.26	$1.46 \times 10^5 \pm 0.08$ / $1.65 \times 10^5 \pm 0.09$	40.0 / 71.5	34.5 / 17.9	1.89 / 1.20	1.5KGy
P1-7K	$6.81 \times 10^5 \pm 0.41$	108	25.3	3.62	$1.57 \times 10^5 \pm 0.09$ / $1.55 \times 10^5 \pm 0.09$	40.3 / 68.0	33.1 / 19.3	2.12 / 1.19	1.7KGy
P2-3K	$6.34 \times 10^5 \pm 0.44$	103	-	3.60	$1.51 \times 10^5 \pm 0.10$ / $1.54 \times 10^5 \pm 0.10$	36.5 / 67.5	36.5 / -	2.07 / 1.23	2.3KGy
P2-8K	$6.60 \times 10^5 \pm 0.45$	101	21.2	3.63	$1.67 \times 10^5 \pm 0.11$ / $1.62 \times 10^5 \pm 0.10$	33.5 / 68.3	30.9 / 14.1	2.10 / 1.23	2.8KGy
P3-8	$5.23 \times 10^5 \pm 0.40$	91.5	-	3.22	$1.33 \times 10^5 \pm 0.10$ / $1.50 \times 10^5 \pm 0.11$	28.8 / 63.2	30.3 / -	1.91 / 1.24	3.8KGy
P5-9A1	$6.00 \times 10^5 \pm 0.14$	91.8	-	4.25	$1.72 \times 10^5 \pm 0.04$ / $1.71 \times 10^5 \pm 0.55$	26.2 / 67.9	37.3 / -	1.97 / 1.40	5.9KGy
P24-8A1	$4.03 \times 10^5 \pm 0.12$	85.4	31.8	4.59	$1.68 \times 10^5 \pm 0.04$ / $1.16 \times 10^5 \pm 0.05$	15.5 / 72.5	37.7 / 30.5	1.97 / 1.60	24.8KGy
P49-8A1	$1.77 \times 10^5 \pm 0.12$	69.5	-	3.43	$1.48 \times 10^5 \pm 0.10$ / $7.62 \times 10^4 \pm 0.58$	4.24 / 63.8	32.1 / -	1.97 / 1.62	49.8KGy

Hyaluronan and the hylan family of cross-linked derivatives

First hyaluronan (HA) was subjected to the radiation processing using up to 3.8 kGy. There is an increase in Mw followed by gel formation in hyaluronan system. Figure 10 a gives the shear viscosity profile for hyaluronan irradiated for various doses. The elastic and flow properties of linear polymeric molecules are attributed to the length of the molecules, the flexibility of the molecular chains and the interactions of the segments of a polymer molecule with other segments of the same and other polymer molecules. From Figure 10a it can be seen that a dose of 2.0KGy produced higher molecular weight molecules that resulted in increasing the shear viscosity of HA system, although some gel particles are clearly present. However, upon increasing the radiation dose to 4.0KGy the high molecular weight hydrogel gel particles increase in size and this can be done in a controlled manner. As a consequence a reduction in shear viscosity was observed. The elastic and viscous response to small oscillatory deformation further confirm the above observation which shows the increase in G' in Figure 10b.

Hylan is a cross-liked derivative of the linear hyaluronan and increases in intrinsic viscosity can be achieved. The doses used were in the same range (0.828 to 4.435 kGy).

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Table 11. Intrinsic viscosity and viscosity average molecular weight of hylan after radiation processing.

Hylan	Intrinsic viscosity (cc/g)	MW(K=0.397) a= 0.601)
Zero kGy (Control)	3926	4.4 (x 10^6)
0.828	5062	6.8
2.484	4684	6.0
3.6	4945	6.5
4.435	4712	6.0

There were no significant new chemical groups introduced by the process, as demonstrated by the fact that infra-red scans were identical for the control and hylan irradiated to 0.5, 1.0 and 2.0kGy (Figure 10c). There was no difference either in the ultraviolet spectrophotometric scans of the control and processed hylan samples. Thus no changes had been introduced into the base structure by the radiation processing. When hylan of high initial molecular weight (6×10^6) is processed, even after a dose of 1.0 kGy, a hydrogel is produced. The volume and particle diameter of the gel particles are shown in Table 12. The spectrum of particle sizes shows that after 2.0kGy 755 of the gel particles were greater than 335µm and 505 were greater than 673µm. The size of the gel particles, and the quantity produced can be controlled by varying the processing conditions.

Table 12. Production of hydrogel after radiation processing of hylan

Sample No.	75% particles > (μm)	50% particles > (μm)
12 / R (Control)	No measurable particles	No measurable particles
0.5Gy	No measurable particles	No measurable particles
1.0Gy	279	552
2.0Gy	335	673

Gelling polysaccharides: Xanthan, Pectin and Carrageenan

The modifications already demonstrated for other groups of polysaccharides can be applied also to the widest range of commercial polysaccharides, including gellan, welan, guar gum, locust bean gum, algin, starch, heparin, chitin and chitosan. Since another of our objectives is to modify and improve gelation characteristics, the well established polysaccharide gelling agents have been selected to illustrate the universality of the process.

Xanthan

The chemical structure of xanthan comprises a (1→4) linked β-D- glucan (cellulosic) backbone substituted through position 3 on alternate glucose residues with a charged trisaccharide side chain. Dispersions of this bacterial polysaccharide exhibit weak gel-like behaviour. This structure is disrupted upon shearing. At low shear rates the viscosity is high and the samples show reversible shear-thinning behaviour. Insensitivity to pH, temperature and ionic strength can be achieved by appropriate choice of experimental conditions.

It is a general practice to induce the gelation of xanthan by the addition of divalent and trivalent inorganic ions such as borate ions. With the process here described it is not necessary to add such chemical additives to achieve gelation, and the process can be achieved with "clean" xanthan in a controlled fashion. Not having foreign additives in the system, of course, greatly increases the range of applications possible using the xanthan alone. Figures 11 a, b, and c demonstrate how the storage, loss moduli and dynamic viscosity, can be enhanced at will over a 10 to 100 fold range without the addition of any inorganic ions. The proportion of gel to viscous component can be varied as required.

Carrageenan and alginates

Alginates and carrageenans are the major polysaccharide components of certain species of marine brown and red algae. Both are linear polymers but quite

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different in primary structure. The carrageenans are based on a disaccharide repeating sequence of alternating 1,3-linked-β-D-galactose and 1,4-linked α-D-galactose, with varying extents and patterns of sulphation. Alginate is a 1,4-linked block co-polymer of β-D-mannuronate and α-L-guluronate. With residues grouped in long, homopolymeric sequences of both types, and in heteropolymeric sequences where the distribution of the two sugars can vary from near random to near alternating. Alginate gives thermally-stable gels with calcium (or larger Group II cations); carrageenan gels melt on heating and reform on cooling. Despite these apparent dissimilarities, however, there are also striking parallels in the way in which the two families of polysaccharides form gel networks.

Both of these gelling polysaccharides can be modified by the process already described to enable the gelling function to be controlled and enhanced. Figure 12 shows and example of kappa-carrageenan shear viscosity profile. Thus a new family of these materials can be produced. Carageenan is produced from naturally occurring seaweed. The molecular parameters are therefore determined entirely by this natural process. Weed from some countries can only yield carageenan and alginate with relatively low molecular weights. The process which we herein describe provides a route to overcome such problems with naturally occurring polysaccharide products.

Pectins

Chemically, pectin is a polyuronide, a straight chain of a few hundred α-D-galacturonic acid molecules linked by 1,4-glycosidic linkages which are all di-equatorial due to the C1 conformation. In carefully extracted pectins from most plants 70 - 80% of the galacturonic acid are methyl-esterified. Pectins are not pure poly-uronides: there are 1,2 - linked α-L-rhamnose molecules interspersed in the galacturonan chain interrupting its conformational regularity by kinks. In carefully extracted pectins from many fruits and vegetables there are 1 to 4 molecules rhamnose and 10 to 15 molecules of arabinose and galactose per 100 molecules of galacturonate. The L-arabinose and D-galactose molecules are covalently bound to rhamnose molecules as complicated side chains. High methoxyl pectins will only gel in the presence of sugars or other co-solutes and at sufficiently low pH, so that the acid groups in the polymer are not completely ionised. Both gel strength and setting temperature are influenced by these factors. In a system with sucrose at around 65% soluble solids, high methoxyl pectins gel up to pH 3.4 (rapid set pectin) or 3.2 (slow set pectin). As the pH is reduced, gel strength and setting

temperature will increase up to the point at which the setting temperature approaches the temperature at which the gel is deposited. Below this pH, pectin tends to pre-gel. Control of the gelling function is therefore important. We have utilised orange pectin to demonstrate that the molecular weight and gelation can be controlled as demonstrated by the increase in G' given in Figure 13. Thus a completely new range of pectin materials can be produced, greatly extending the applicability.

Example 3

Protein Systems

Gelatin was selected to demonstrate the applicability of the process to connective tissue proteins as well as carbohydrate systems. This series of materials does not exist in nature and are derived from the parent collagen by processes that destroy the secondary and higher structures with varying degrees of hydrolysis of the polypeptide backbone. The main source is the skin or bones of animals. This protein has a high content of glycine, proline and hydroxy-proline, with a structure which involves glycine – proline-hydroxy-proline triplets. Its triple helical structure allows it to form gels on heating and cooling in water. Although at temperatures above 35 to 40 °C gelatins in solution behave as random coils, which can take up an infinite number of transient configurations. On cooling the solution, aggregation occurs and at concentration above about 1% depending on the quality of the gelatin and pH, a clear transparent gel will form. Unlike most protein and polysacharide gels, gelatin gels are thermoreversible, since on warming the gel will dissolve. Using the solid state process described hereinabove, the molecular weight can be increased in a controlled manner to produce a range of products with varying molecular weights and solution/gelling properties (Figs. 14a, b and c.)

To demonstrate the generality of the phenomenon also with other proteins the same behaviour was enacted with casein in the form of its sodium salt.

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Example 4
Interactive Blends

5   A basic technology associated with hydrocolloids is to develop synergistic blends
which have a different rheology from the simple additive behaviour of each component,
usually a higher viscosity than the sum of the parts.

The process of the present invention enables such synergy to be greatly extended and
10   provides an increased enhancement of viscosity and viscoelasticity compared with
separately prepared solution mixes by several orders of magnitude. The co-processing
of simple solid state mixtures using the technology developed achieves closer
interaction of newly formed higher molecular weight forms of the individual
components. The rheological behaviour cannot be reproduced by simply preparing
15   selected mixtures of the parent materials.
Specific examples of these blends are:
Carboxymethyl cellulose - pectin systems (Fig 15)
Pectin-dextran - systems (Fig 16)
Carboxymethyl cellulose - dextran (Fig 17)

20   The dynamic viscosity, loss and storage modulus of processed blends of these systems
can be increased in a controlled manner, and presented for use in a convenience and
cost-effective mixture form.

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Example 5

Adhesion and Bonding

When blends of polysaccharides or polysaccharide-proteins are prepared in a closely interactive state, binding of the components can be achieved. The rheological behaviour of the resulting products can be controlled such that the viscoelasticity is either higher or lower than the corresponding mixtures of the components given the identical processing treatment singly. New product formation incorporating the two components can be produced. It is possible also to interact and similarly co-process mixed systems of natural polysaccharides with water soluble synthetic products.

As illustrative examples of producing the effects identified we cite the following blends produced in the closely interactive state:

(a) when lower composite viscoelasticity can be produced
   polyvinyl pyrrolidone – dextran (Figure 18)

The closely interactive blend shows a lower dynamic viscosity (after 10 kGy) than the components processed separately. Polyethylene oxide – arabinogalactan protein interactive blends were used to demonstrate the same behaviour using different types of structural components.

(b) when a higher composite viscoelasticity can be produced
   polyviny pyrrolidone – arabinogalactan protein

The closely interactive blend after processing has several orders of magnitude greater viscoelasticity than the equivalent mixture of the components processed singly, when this itself is several orders of magnitude greater than the unprocessed controls (Figure 19).

(c) when a higher viscoelasticity can be produced using only polysaccharides
   carboxymethyl cellulose – arabinogalactan protein blends

The previously reported enhanced rheology for selected closely interactive blends is repeated (Figure 20) and the new product formed can be produced

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such that the proportions of the individual components in the composite can be varied.   Figure (21) illustrates this behaviour using gel permeation/molecular weight distribution behaviour.

(d) when higher or lower viscoelasticity can be obtained according to the selected conditions

dextran − pectin

According to processing conditions, the final closely interactive state can be used to form a composite with either lower or higher viscoelasticity than the same blend of the individually processed components (Figure 22), which is itself orders of magnitude greater viscosity than the unprocessed control mixtures.

The new products so produced can be prepared in forms which adhere to cellulosic surfaces to provide enhanced biocomptibility, while retaining complete biodegradability. When prepared on the surface of such systems, the adhesion is greater, with some indication of some form of close bonding.

Uses

The use of closely interactive blends processed in the manner described can produce a range of new composites. Products so produced are different in behaviour from the synergistic blends described in Example 4. These new composite matrices can have either lower or higher viscoelasticity than the conventionally processed mixtures. Thus a new range of products can be developed either as gels, viscous solutions or as membranes. Thus these can be designed for specific applications as medical membranes and pharmaceutical excipients, drug delivery systems and as carriers for the delivery of wound-healing cell signalling macromolecules such as hyaluronan and alginate.

Example 6

Enhancing the Biological Function of Demineralized Bone
(DMB) in Building New Bone Formation

Background

Human bone, when treated by a process for the differential removal of bone mineral to give "demineralized bone" (DMB), has the capacity to actively induce new bone

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growth when transplanted into humans (J.N.Kearney and R J. Lomez, Advances in
Tissue Banking, 1997, 1, 43 – 71). Such material has a wide application in oral and
maxillofacial surgery, since the osteoinductive capacity of such allogenaic bone allows
the transformation of the primitive mesenchymal precursor cells into chondroblasts or
osteoblasts, (C.J.Yim, Advances in Tissue Banking, 1999, 3, 87 – 111). The process
of the present invention permits one to transform DMB into a material with greatly
enhanced osteoinductive activity, which accelerates and improves the quality of new
bone formation.

Materials and Methods
    1. Experimental animals
Ninety healthy male white rats (250~300g) were used for this experimental study. The
animals were divided in the three groups: negative control, control (using DMB), and
experimental (using new processed bone – NPB)). The rats were housed (5 rats in
each cage) in the standard circumstance of the Experimental Animal Room at the
School of Dentistry, Dankook University, Republic of Korea. They were fed a solid
diet during the experimental periods. After 2 weeks adaptation periods, the animal
experiments were started.
    2. Manufacturing of DMB and NPB
 Healthy male rats were sacrificed by cervical dislocation in order to obtain the femurs
and tibias of each rat. After procuring the bones, they were kept in deep freezer at –70°
C) for 24 hours and thereafter crushed to fine particles using a bone mill. The bones
were then processed as follows : The particles were stirred in distilled water 6 times
repeatedly every 30 min., demineralized in 0.5N hydrochloric acid for 5 hours,
followed by 4 washings in sterile water for 2 hours, soaked in absolute ethanol for l
hour at room temperature (25° C), further washed in distilled water, for 3 hours, soaked
in diethylether for 30 min and dried in vacuo over night.
After these procedures the DMB particles were was divided into various sizes using
appropriate sieves. Only 350-600 μm sized DMB particles were used in this study.
The DMB was subjected to the gas mediated – radiation processing treatment described
above after which they are as designated "New Processed Bone" (NPB). After triple
packaging all samples of DMB and NPB were sterilized with 1.5 Mrad γ-radiation.

    3. Methods (Surgical Procedures)
1) Anesthesia
Animals were injected preoperatively with Oxytetracycline and then anesthetized with
Ketamine Hydrochloride (intra-muscularly, 10mg/kg) and Xylazine Hydrochloride

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(0.15ml/kg).

2) Surgery:

In the usual manner, after calvarial flaps were reflected, bone defects were created around the middle calvarium with Ø6mm trephin-burr and a low-speed dental drill. During formation of defects with the burr, cooling water (sterilized saline) prevented the operating site from overheating. Then the three groups of animals were subjected to the following procedure:

2.1. Negative control group: the wounds were closed without any graft materials

2.2. Control Group: these were grafted with pure DMB (15mg/each)

2.3. Experimental Group: these were grafted with NPB (15mg/each)

       For all groups closure was performed using 4-0 Vicryl, layer by layer.

       All the above procedures were performed aseptically.

3) Sacrificing the animals

After surgery five animals from each group were sacrificed within 1, 2 and 3 weeks respectively, and then their defect site (in the calvarium) removed with full thickness flap, including periosteum. The sections of the calvarial were fixed in 10% neutralized formalin.

4. Fabrication of the specimen

After fixation of the calvarium in 10% neutralized formalin, the bone defects were decalcifying for 3 days with 5% nitric acid. In the usual manner, dehydration and cleaning were performed, after which. the calvarium samples were embedded in paraffin. The paraffin sections were stained and examined under a microscope for bone formation.

5. Histologic analysis confirmed the bone formaqtion observations.

6. Measuring the strength of the interbony-union.

Full-thickness of calvarium, including the defect site, were obtained from the animals at each interval and tested for the strength of the inter-bony-union.

The bone specimens were fixed to the holder of a Universal Testing Machine. The rate of compression of testing machine was 5mm/min. The arm section of the testing machine was round (diameter 1mm).

Result (the bone strength in MPa)

	DMB	NPB
1 Week	2.732386	2.873096
2 Weeks	2.823129	4.372102
3 Weeks	2.848112	8.994038
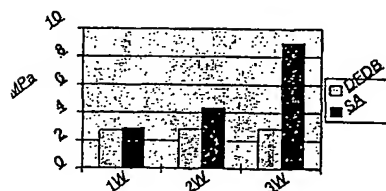
Conclusion

The processed demineralised bone (NPB) is more effective than the control in creating new bone throughout the healing of the bone, and after three weeks the bone is more than 3 times stronger than is the control. This is a significant advance in enhancing bone growth and should have wide ranging applications in oral and maxillofacial surgery, where demineralized bone is routinely used. The potential applications also include orthopedic use, and offer a new approach to the healing of transplanted bone after trauma or disease. Modification of soft connective tissues can also be accomplished in a similar manner to introduce new and enhanced functional properties.

Representative Uses of the New Product Category Representations

1.   Arabinogalactan Proteins

Emulsification of oil in water systems for application as beverages, oils, emulsions, lotions for cosmetic, medical and pharmaceutical use.

New adhesives for paper and related cellulosics.

High molecular weight fibers to enhance bacterial fermentation in the colon for cholesterol and diabetic control.

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Gels which can be applied as gelatin replacements, microcapsules, photographic film and as microbiological media and in tissue culture.

5   Acacia gels for excipient, control release of pharmaceutical/medical active agents.

Membranes for delivery of wound healing active agents by greater water binding characteristics.

10   2.   Polysaccharides

A standard range of molecular weight standards for gel permeation and gel electrophoresis.

New range of blood plasma substitutes

Soluble and insoluble polysaccharide systems for immobilization of proteins, enzymes, mammalian cells, cofactors and drugs.

New gels, membranes and films for medically-related applications.

A new generation of polysaccharide fibers for application as food additives and ingredients. The molecular weight control greatly extends the applicability within a whole range of food products, as thickeners, gelling agents, stabilizers, flavor pseudoplasticizer and ingredient encapsulators.

A new carboxymethyl cellulose ingredient for the baking industry, to give better dough stability and excellent resistance to dough softening.

A new generation of materials to thicken water systems, surfactants suspend solids in aqueous media, retard crystal growth, absorb water from the atmosphere form films, and as plastics additives and packaging.

A range of membranes for chromatography with varying charge, chiral and ligand interactive properties.

A new range of pourable gels, improving gelatin characteristics.

Industrial applications as metal chelating polysaccharides for applications ranging from waste water treatment to precious metal recovery and metal chilate chromatography.

3.   Protein - polysaccharide systems

Application of the new material products as wound dressings, adhesive pads for surgical use, hemostatic agents, blood intradermal augmentation, controlled release drug delivery systems for topical

and oral administration.

All existing applications of biocompatible hydrogels can be extended using the process modified materials and the new adhesion discovery for such applications as coatings for medically related polymer equipment, vascular grafts and sue such as for contact lenses, artificial corneas, soft tissue augmentation, etc.

4.   Tissue Modification

Tissues derived from human and animal origin, such as bone, skin, tendon, cartilage can be endowed with greatly improved physical and biological performance when used as grafts or transplants. Demineralized bone can be endowed with up to four times greater bone healing characteristics after the processing described. Such bone has extensive applications in orthopedic, oral and maxillofacial surgery to promote new bone growth after bone loss through trauma or disease.

<u>Claims</u>

1.   A process for modifying a naturally occurring biocompatible biopolymer, said process comprising subjecting said biopolymer, in the solid, or dry state, to a source of ionizing radiation in the presence of a mediating gas and annealing the resulting product in the absence of oxygen at a temperature of about 40 to 120°C, and thereafter removing any residual mediating gas.

2.   A process according to Claim 1 wherein the source of the ionizing radiation is a γ-ray emitting radioactive isotope, X-rays or high energy radiation generated by an electron accelerator.

3.   A process according to Claim 2 wherein the dose of ionizing radiation to which the biopolymer is subjected to is from about 1 to 50 kGy.

4.   A process according to Claim 2 wherein the radioactive isotope is ^{60}Co.

5.   A process according to Claim 2 wherein the radiation is generated by an electron generator of 250 KeV to 10 MeV capacity.

6.   A process according to Claim 1 wherein the mediating gas is an unsubstituted alkenic or alkynic gas, and is ethylene, propylene or acetylene.

7.   A process according to Claim 6 wherein the mediating gas is acetylene.

8.   A process according to Claim 6 wherein the mediating gas is a mixture of ethylene and propylene.

9.   A process according to Claim 1 wherein annealing is effected in the presence of the mediating gas, an inert gas or in vacuo.

10.   A process according to Claim 9 wherein the inert gas is nitrogen or helium.

11.   A process according to Claim 1 wherein removal of any residual mediating gas is effected by aerating the system and optionally, additionally applying vacuum.

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12.   A process according to Claim 1 wherein the biopolymer is a polysaccharide
       of micro-organism, plant or animal origin, a protein of animal connective
       tissue origin, a protein of other animal tissue origin, a combination of at
       least one of said polysaccharides and at least one other protein of plant
       origin, or demineralized bone ("DMB").

13.   A process according to claim 12 wherein the biopolymer is a polysaccharide
       of plant origin.

14.   A process according to claim 13 wherein the polysaccharide is dextran
       pullulan xanthan, carageenan or pectin.

15.   A process according to claim 12 wherein the biopolymer is a polysaccharide
       of animal origin.

16.   A process according to claim 15 wherein the polysaccharide is hyaluronan,
       or a proteoglycan.

17.   A process according to claim 12 wherein the biopolymer is a protein of
       plant origin.

18.   A process according to claim 17 wherein the protein is an arabinogalactan.

19.   A process according to claim 18 wherein the arabinogalactan is an acacia
       plant exudate.

20.   A process according to claim 12 wherein the biopolymer is a protein of
       animal connective, or other tissue origin.

21.   A process according to claim 20 wherein the protein is of connective tissue
       origin and is collagen or gelatin.

22.   A process according to claim 20 wherein the protein is casein.

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23. A process according to claim 12 wherein the biopolymer is a combination of at least one said polysaccharide and one said protein.

24. A process according to claim 12 wherein the biopolymer is demineralized bone.

25. A process according to claim 1 wherein the biopolymer to be modified is characterized by the absence of any functionalizing groups.

26. A process according to claim 1 wherein the modified biopolymer retains the same degree of biocompatibility as the starting material.

27. A process according to claim 1 wherein the modifications to the treated biopolymer include (a) increasing the molecular weight to endow the biopolymer with increased emulsifiability and water binding ability. (b) endowing the biopolymer, when in aqueous solution, with the ability to achieve a desired degree of viscosity or viscoelasticity; and (c) enabling the biopolymer to be converted into hydrophilic gels of predetermined size and micromechanical properties.

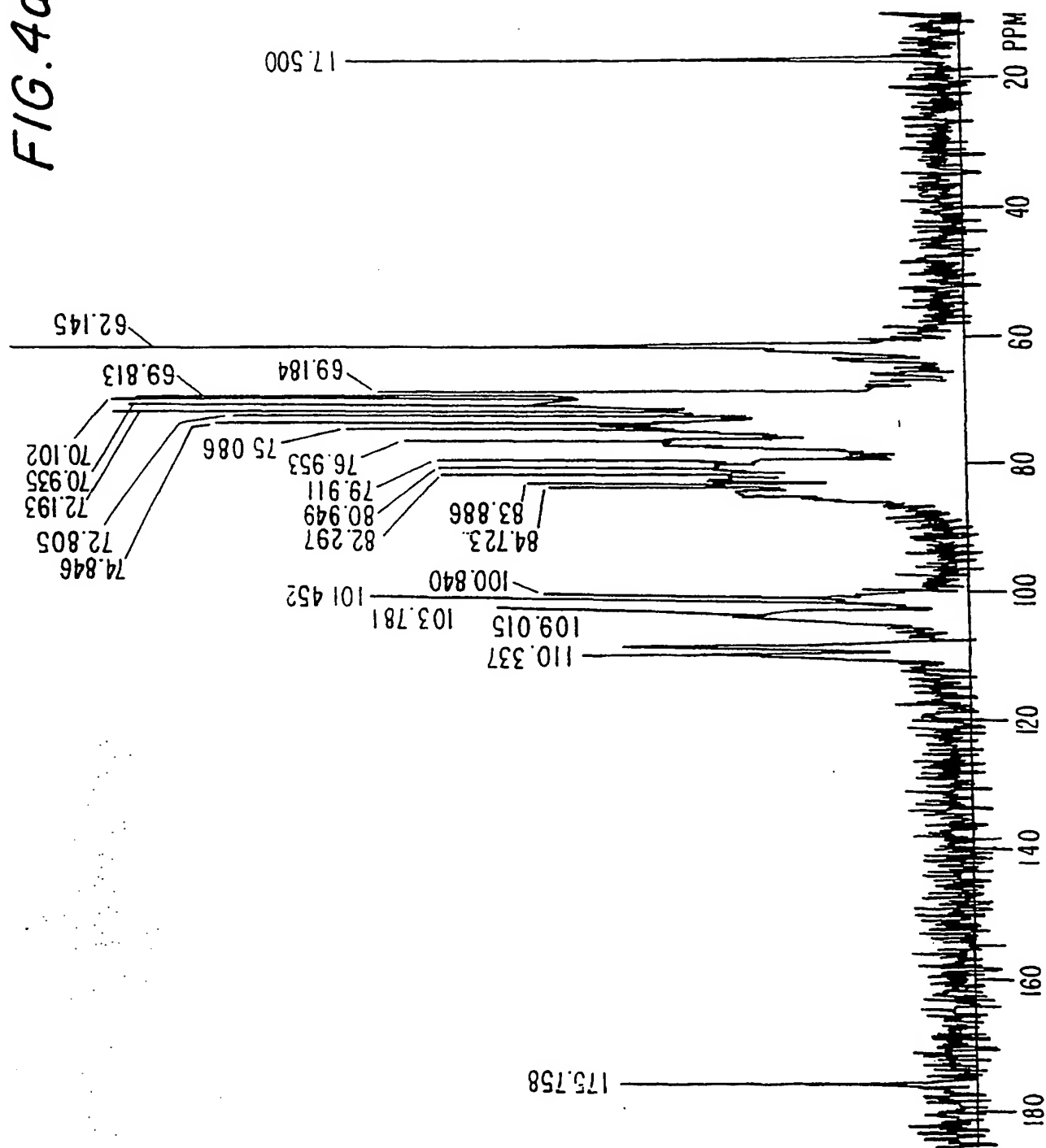28. A process according to claim 1 wherein the biopolymer to be modified is carboxymethyl cellulose ("CMC"), a mixture of CMC and dextran or a mixture of CMC and pectin.

29. A process according to claim 1 wherein the biopolymer to be modified further includes polyvinyl pyrrolidone ("PVP").

30. A process according to claim 29 wherein the bioplymer is a mixture of PVP and dextran or an arabinogalactan.

31. A process as claimed in claim 1 wherein the process is conducted at a pressure which varies to permit maximal penetration of the mediating gas into the polymer to thereby optimize the exposure of the treated material to the irradation during the reaction.

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32.   A process for modifying a tissue of animal origin, said process comprising subjecting said tissue sample or a component thereof in the solid, or dry state, to a source of ionizing radiation in the presence of a mediating gas and annealing the resulting product in the absence of oxygen at a temperature of about 40 to 120°C, and thereafter removing any residual mediating gas.

33.   A process according to claim 32 wherein tissue is bone ray emitting radioactive isotope, X-rays or high energy radiation generated by an electron accelerator.

34.   A process according to claim 33 wherein the bone is whole bone or demineralized bone.

35.   A process according to claim 34 wherein the tissue is soft tissue.

36.   A modified biopolymer produced by the process as claimed in claim 1.

37.   A modified naturally occurring biocompatible biopolymer selected from the group consisting of a polysaccharide of plant or animal origin, a protein of animal connective tissue origin, a protein of other animal tissue origin, a combination of at least one of said polysaccharides and at least one other protein of plant origin, or demineralized bone ("DMB") and having the same degree of biocompatability as the unmodified biopolymer, wherein the modifications include:

          an increase in the molecular weight which enables the biopolymer to be emulsified and to bind with water to a greater extent than the unmodified biopolymer;
          changed, to a predetermined extent, viscosity or viscoelasticity; and conversion to a hydrophilic gel of predetermined size and micromechanical properties.

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FIG. I

FIG. 2a

FIG. 2b

FIG.3

FIG.4a

FIG. 4b

FIG.5

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FIG. 6a

▲G' Pa / Control   ▫G' Pa /2.3K   ◆G'Pa/2.8K
○G' Pa / 3.8K   ◇ G' Pa/ 6.1K   ■G'Pa/10.5K
                        ▫G' Pa/13.9K

FIG. 6b

▲ G'' Pa/control   ▫ G'' Pa / 2.3K   ◆G''Pa/2.8K
○ G'' Pa/ 3.8 K   ◇ G''Pa/ 6.1K   ■G'' Pa /10.5K
                        ▫ G''Pa/13.9K

FIG. 7a

FIG. 7b

CONTROL ● 1.5k + 1.7k △ 2.3k
■ 2.8k □ 3.8k ▲ 16.2k × 24.8k

FIG.8a

FIG. 8b

○ CONTROL   □ 1.5KGy   △ 2.8KGy

FIG. 8c

○ CONTROL   △ 1.5k   □ 2.8k

P-C2
NORM-LOG
1ST ORDER
P1-2A1
NORM-LOG
1ST ORDER
P1-5K
NORM-LOG
1ST ORDER
P1-7K
NORM LOG
1ST ORDER
P2-3K
NORM-LOG
1ST ORDER
P2-8K
NORM-LOG
1ST ORDER
P3-8K
NORM-LOG
1ST ORDER
P5-9A1
NORM-LOG
1ST ORDER
P24-8A1
NORM-LOG
1ST ORDER
P49-8A1
NORM-LOG
1ST ORDER

MOLAR MASS (g/mol)

DIFFERENTIAL WEIGHT FRACTION

1.2

0.8

0.4

0.0

1.0X10

FIG.9a

FIG. 9b

○ G' Pa / Control   ● G' Pa/ 2.3K   ■ G' Pa / 2.8K

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FIG.10a

FIG.10b

FREQUENCY (HZ)

G"(Pa)

2.8kGy
1.5kGy
CONTROL

FIG.10c

CONTROL 12/R

12/PRE

12/RY

12/RA

FIG.11a

○ G'/control   ● G'/0.7kGy

FIG.11b

○ G"/control   ● G"/0.7kGy

FIG.11c

○ n'/Control   ● n'Pa.s/0.7kGy

FIG. 12

○ CONTROL   ● 16.2 k Gy   ▲ 24.8 k Gy

FIG. 13

△ G'/control   ● G'/3k

FIG. 14a

○ G'/control　　●G'/3k　　△G'Pa/10k

FIG. 14b

○G''/control　　●G''/3K　　△G''Pa/10k

FIG. 14c

○n'/control　　●n'/3k　　△n'Pa.s/10k

FIG.15

OCONTROL　　　△3.3k Gv　　　□10.4 kGy

FIG. 16

OCONTROL　　　●3.3kGy

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FIG.17

FIG.18

FIG. 19a

○CONTROL   ●3k   △3M1   ■10K   □10M1

FIG. 19b

○CONTROL   ●3k   △3M1   ■10K   □10M1

FIG. 19c

○CONTROL   ●3k   △3M1   ■10K   □10M1

FIG. 20a

△ CONTROL   ● 3.1kGy- PROCESSED TOGETHER
                       ○ 3.1kGy- PROCESSED SEPARATELY

FIG. 20b

△ CONTROL   ● 3.1kGy- PROCESSED TOGETHER
                       ○ 3.1kGy -PROCESSED SEPARATELY

FIG. 20c

△ CONTROL   ● 3.1kGy-PROCESSED TOGETHER
                       ○ 3.1kGv-PROCESSED SEPARATELY

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FIG. 22a

G'(Pa) vs FREQUENCY(Hz)

△CONTROL   ●3.1kGy-PROCESSED TOGETHER
○3.1kGy-PROCESSED SEPARATELY   ■10.4kGy-PROCESSED
□10.4kGy-PROCESSED SEPARATELY   TOGETHER

FIG. 22b

G''(Pa) vs FREQUENCY(Hz)

△ CONTROL   ●3.1kGy-PROCESSED TOGETHER
○3.1kGy-PROCESSED SEPARATELY   ■10.4kGy-PROCESSED
□10.4kGy-PROCESSED SEPARATELY   TOGETHER

FIG. 22c

DYNAMIC VISCOSITY, η' (Pa.s) vs FREQUENCY(Hz)

△CONTROL   ●3.1kGy-PROCESSED TOGETHER
○3.1kGy-PROCESSED SEPARATELY   ■10.4kGy-PROCESSED
□10.4kGy-PROCESSED SEPARATELY   TOGETHER